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Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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Novel process for enzymatic bleaching of food products

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NOVEL PROCESS FOR ENZYMATIC BLEACHING OF FOOD PRODUCTS

Field of the invention

The present invention relates to a method for preparing a food product having increased whiteness, and the food product obtained.

In some types of food product a white colour of at least part of the food product is seen as desirable, for example in dairy products, for example cheeses, whey, butter, and milk powder and in flour-based products, for example bread and noodles.

The raw materials or intermediate products of such food products however may comprise pigments, which can cause an off-white to yellow colour of the food product. Examples of such pigments are carotenoids (carotenes and xanthophylls) and flavones.

In white bread for example, a white crumb is seen as a desirable property. A whiter crumb may be obtained by using enzymes such as catalase, peroxidase, lipase and/or lipoxygenase, see for instance *'Oxido-reductases and Lipases as Dough-Bleaching Agent'* by P. Gélinas et al, Cereal Chem, 75(6), 810-814 (1998). All enzymes mentioned have a bleaching effect on the crumb. At present, the baking industry mostly uses enzyme active soy flour, which contains lipoxygenases. The lipoxygenases in the soy flour are capable of bleaching wheat flour pigments as a result of the action of free radicals and other reactive oxygen species that are formed during the oxidation of fatty acids by lipoxygenase. This reaction is called a co-oxidation. In soy flour, three lipoxygenases are present, L1, L2 and L3 whereby L2 and L3 possess the best bleaching activity (W. Grosch, G. Laskawy and F. Weber, J. Agric. Food Chem 24 (1976), 456).

Soy flour not only contains lipoxygenases but also the fatty acids that are necessary for the bleaching effect, resulting in an improved bleaching effect.

A disadvantage associated with the use of soy beans as source of lipoxygenase, is the fact that nowadays most of the soy beans are genetically modified (GMO). Since there is a world-wide consumer preference for using non-GMO derived bread improving additives, an alternative for the soy lipoxygenases is highly required. The known enzymes other than the lipoxygenases L2 and L3 from soy have the disadvantage that their performance is not as good as the lipoxygenases from soy. In practice, to obtain the

desired whiteness, these enzymes are to be combined with cofactors or other enzymes to reach the desired level of whiteness of the crumb. Peroxidases catalyze non-enzymatically the oxidation, by molecular oxygen, of unsaturated compounds e.g. unsaturated fatty acids. (C.E. Eriksson et. al. JAOS 48 (1971) 442). These oxidized fatty acid generate radicals that probably react with flour pigments to less coloured products in a similar way as the lipoxygenase reaction products.

It is the object of the present invention to provide a novel food product having increased whiteness of at least part of the food product. This object is reached by a novel process for the production of a food product in which an intermediate form of said food products comprises a pigment, which process comprises adding at least one enzyme that is effective in directly converting said pigment into a form which results in increasing the whiteness of at least part of the food product compared to the food product for which said enzyme is not added during its production.

Enzymes capable of directly converting pigment into a form which results in increasing whiteness are here and hereafter referred to as bleaching enzymes. These enzymes can in various ways exert their direct bleaching effect on the pigments. For example, they can directly convert the pigments by saturating unsaturated bonds in the pigments via for example hydrogenation, or they can directly cleave the pigments, forming degradation products. With the term direct is meant that these enzymes act upon the pigment as substrate itself, not by first producing a compound which then acts on the pigment. Use of co-factors for reaching the conversion is not specifically excluded.

Enzymes capable of directly cleaving pigments will here and hereafter be referred to as cleaving enzymes. Suitable cleaving enzymes according to the invention are enzymes that are capable of cleaving carotenoids (carotenes and xanthophylls) and flavones. Carotenoids can be cleaved in two different ways, central and eccentric. Central cleavage of carotenoids results in formation of retinoids (C_{20}-compounds). Eccentric cleavage can yield a more diverse group of compounds, as for example abscisic acid. An enzyme capable of central cleavage of carotenoids is for example β-carotene 15,15'-monooxygenase (E.C. 1.14.99.36). An additional advantage of the use of enzymes capable of central cleavage is the formation of retinoids. These are essential components in vision. β-carotene is cleaved into two molecules of retinal. This retinal can be modified to retinol, also known as vitamin A. Examples of enzymes capable of eccentric cleavage of carotenoids are 9-cis-epoxycarotenoid dioxygenase and β-

carotene 9',10'-dioxygenase.

An intermediate form of the food product is defined herein as any form that occurs during the production process prior to obtaining the final form of the food product. The intermediate form may comprise the individual raw materials used and/or mixture thereof and/or mixtures with additives and/or processing aids, or subsequently processed form thereof.

The food product may be made from at least one raw material that is of plant origin, such as wheat flour. The latter is known to contain pigments such as carotenoids (carotenes and xanthophylls) and flavones, which are responsible for, for example, the crumb colour of baked bread.

Alternatively, these pigments may originate from other sources than plant raw materials e.g. from milk.

A preferred food product for the process according to the invention is baked bread and other baked products from wheat flour and/or flours from other cereal origin.

For example, for the baked food product bread, the intermediate forms comprise for example wheat flour, the initial mixture thereof with other bread ingredients such as for example water, salt, yeast and bread improving compositions, the mixed dough, the kneaded dough, the leavened dough and the partially baked dough.

In some types of noodles, a white product is seen as desirable. For example, for noodles, the intermediate forms comprise for example wheat flour, the initial mixture thereof with water, salt, and other noodle ingredients, the mixed dough and the final noodle product that can be fresh, dried, boiled, steamed and/or fried.

The food product can also be a dairy product. By dairy products is meant products that contain at least 10 wt%, preferably at least 30 wt%, more preferably at least 50 wt%, still more preferably at least 70 wt% or most preferably at least 80 wt% on dry solid basis of components originating from milk, preferably cow's milk. Components originating from milk are for example fats, proteins, for example whey cheese curd and casein, etc. Milk, especially cow's milk, may naturally contain colouring compounds such as carotenoids, for example β-carotene.

Whiteness plays an important role in for example cheese, butter oil, milk powder or whey products. For example for cheeses like Feta, Mozzarella, Ricotta and blue cheese, for example Danish Blue, Roquefort or Gorgonzola, whiteness is considered desirable. In cheeses wherein milk from goat or sheep is at least partially replaced by

cow's milk, the whiteness of the cheese might be a problem because of the β-carotene that is present in cow's milk.

For some cheeses natural colouring agents like annatto or β-carotene are used as food colouring agents. However, this colouring agent will also be present in the whey. When this whey is further processed into for example baby formula, the colour of the whey product may be undesirable.

For the food product soft cheese, the intermediate products comprise e.g. milk, and cheese curd.

The enzyme may be added as an enzyme preparation or produced in situ by a microorganism capable of producing said enzyme. The enzyme preparation can be derived from various sources, for example from plants, animals and microorganisms. Preferably the enzyme preparation is derived from a microorganism, since microorganisms make it possible to obtain the enzyme on an industrial scale in a controlled manner. The enzyme preparation derived from a mircroorganism can be obtained by classical fermentation processes of a selected microbial strain or by fermentation of a microorganism that overexpresses the enzyme. The microorganism may be a bacterium, a fungus or a yeast. Examples of suitable microorganisms are *Microcystis*, *Lepista*, for example *L. irina*, *Cyathus*, for example *C. pallidus*, *Ganoderma*, for example *G. applanatum*, *Ischnoderma*, for example *I. benzoinum*, *Marasminus*, for example *M. scorodonius*, *Trametes*, for example *T. suaveoluens* of *T. versicolour*, *Cryptococcus*, for example *C. laurentii*, *Hypomyces*, for example *H. odoratus* or *Phaffia*, for example *P. rhodozyma*, *Phanerochaete* for example *P. chrysosporium*, *Lentinula* for example *L. edodes*, *Coprinus* for example *C. cinereus*, *Gloeophyllum* for example *G. trabeum*, *Ophiostoma* for example *O. piliferum*, *Aspergillus* for example *A. niger*, *A. oryzae*, *A. nidulans*.

Measurement of whiteness of a product can be done visually or a reflection measurement, for example by scanning. In reflection measurement the colours are quantified with three parameters: L-factor(black =0 to white= 100), a-factor (green =-60 to red = +60) and b-factor (Blue = -60 to Yellow = +60). In case of carotenoids, the b-factor of the produced product is preferably as closest to 0 as possible, preferably between 10 and 0, more preferably between 5 and 0 and even more preferably lower than 1 and most preferably lower than 0.5.

In a second aspect, the invention provides a food product obtainable by the process of the invention as described hereinbefore. These food products are characterized by at least parts having significantly increased whiteness in comparison with food products obtainable by production processes do that do not comprise adding one or more of enzymes capable of converting pigments in the intermediate products.

In a further aspect, the invention provides the use of enzymes capable in converting pigments for bleaching food products, for example flour-based or milk-derived products. Surprisingly, it was found that these enzymes can advantageously be used as a stain remover in household detergents. In particular, the enzymes proved very efficient in removing coloured stains, for example grass stains, coffee and tea stains, from both cotton and synthetic (e.g. polyester) fabrics. Furthermore, the enzymes could also be used in enzymatic stone bleaching processes, for example by bleaching the indigo dye of blue jeans to a desired level.

Materials and methods

Bleaching was determined after extraction of carotenoids from crumb or dough as indicated by Gelinas, Cereal Chem. 75, 810-184 (1998).

Carotenoids were determined as indicated by Gelinas, Cereal Chem. 75, 810-184 (1998) via total lipids extraction from crumb of bread.

Enzyme activity was determined as β-carotene degradation activity according to A. Ben Aziz, Phytochemistry 10(1971)1445. 1 unit of enzyme is the amount of enzyme that degrades 1 microgram of ß-carotene /min.

Whiteness of a food product can be determined both visually as well as by reflection measurements. Visual inspection can be performed by comparing food products to which a bleaching enzyme is added versus a control without added bleaching enzyme. Reflection measurements can be performed by scanning the food product on a colour scanner (Hewlett Packard Scanjet ADF). These data can be analysed using the programme LabSMART (LabSMART, LLC, Logan Utah, USA).

The used bleaching enzyme was obtained from *Marasminus scorodonius* (enzyme I) or *Aspergillus niger* (enzyme II).

Example I and comparative examples A, B and C

Pup loaf baking test

Preparation of pup loaves in a standard baking process was done by mixing 200 g of wheat flour, 1.6 g Fermipan dry yeast, 3 g salt, 40 ppm ascorbic acid, 5 ppmfungal α-amylase Bakezyme P500, 60 ppm of fungal hemicellulase Bakezyme HS2000, an amount of the enzyme to be tested as indicated in Table 1 and 116 ml water in a pin mixer for 6 minutes and 15 seconds. The dough temperature is 28° C. Directly after mixing the dough is divided into two pieces of 150 g, rounded and proofed for 45 minutes in a proofing cabinet at 30° C, shaped and panned. After a final proof of 70 minutes at 30° C, the dough was baked for 20 minutes at 225° C.

After 24 hrs of storage in a closed box at room temperature the baker assesses crumb quality and colour and the amount of carotenoids is determined after extraction of the bread crumb as indicated in Table II.

Table 1 Enzyme dosage (on 200 gram of flour)

	Loaf A	Loaf B	Loaf C	Loaf I
Enzyme active soy flour	-	10,000 U		-
Soy enzyme L2 (Sigma)	-	-	10,000 U	
Bleaching enzyme I	-	-		10,000 U

Table 2. β-Carotene content of the loaves and visual identification

	Loaf A	Loaf B	Loaf C	Loaf I
% Carotene present	100	8	30	5
Visual inspection	Yellowish	White	Off-White	White

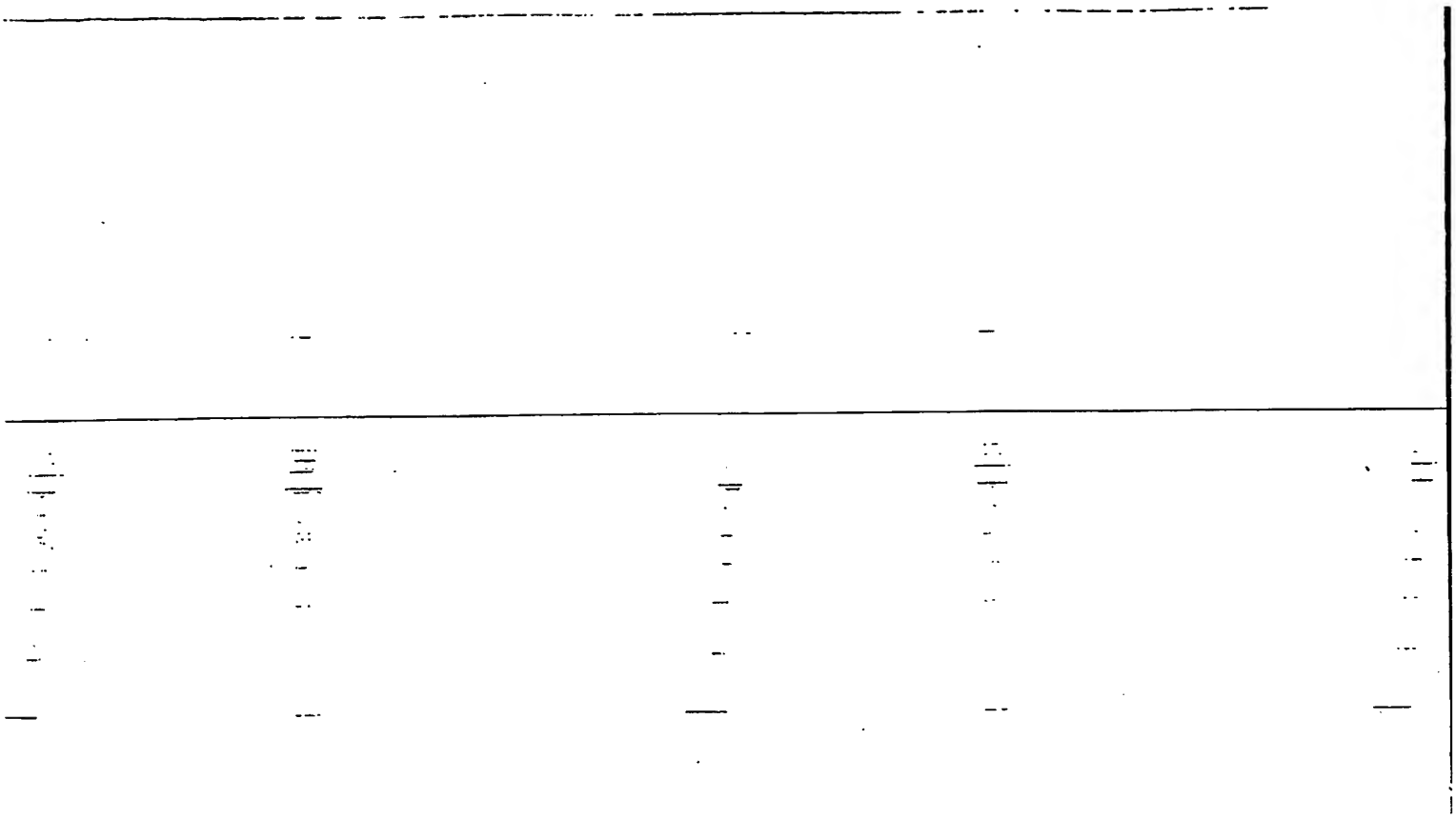
From Table 2 can be concluded that by addition of the bleaching enzyme according to the invention to the dough, carotene is degraded, resulting in a whiter crumb. The efficiency of the process according to the invention is better than for the used soy enzyme L2, and is at least equal to or better than the use of enzyme active soy flour.

Example II

Preparation of mini cheeses

Miniature cheeses were produced as described by Shakeel-Ur-Rehman et al. (Protocol for the manufacture of miniature cheeses in Lait, 78 (1998), 607-620). Raw cows milk was pasteurised by heating for 30 minutes at 63°C. The pasteurised milk was transferred to wide mouth plastic centrifuge bottles (200mL per bottle) and cooled to 31°C. Subsequently, 0.72 ml of starter culture DS 5LT1 (DSM Gist B.V., Delft, The Netherlands) was added to each of the 200 ml of pasteurised milk in the centrifuge bottles and the milk was ripened for 20 minutes. Then, $CaCl_2$ (132 µL of a 1 $mol.L^{-1}$ solution per 200mL ripened milk) was added, followed by addition of the coagulant (0.04 IMCU per ml). In case the experiment involved the use of bleaching enzyme I or II, this enzyme was added together with the coagulant.

The milk solutions were held for 40-50 minutes at 31°C until a coagulum was formed. The coagulum was cut manually by cutters of stretched wire, spaced 1 cm apart on a frame. Healing was allowed for 2 minutes followed by gently stirring for 10 minutes. After that, the temperature was increased gradually to 39°C over 30 minutes under continuous stirring of the curd / whey mixture. Upon reaching a pH of 6.2 the curd / whey mixtures were centrifuged at room temperature for 60 minutes at 1,700*g*. The whey was drained and the curds were held in a water bath at 36°C. The cheeses were inverted every 15 minutes until the pH had decreased to 5.2-5.3 and were then centrifuged at room temperature at 1,700*g* for 20 minutes. After further whey drainage the cheese bleaching was determined by scanning. Use of bleaching enzymes I and II resulted in a whiter cheese.



CLAIMS

1. Process for the production of a food product whereby an intermediate form of said food product comprises a pigment, which process comprises adding at least one enzyme that is effective in directly converting said pigment into a form which results in increasing the whiteness of at least part of the food product compared to the food product for which said enzyme is not added during its production.

2. Process according to claim 1 wherein the food product is made from flour, preferably wheat flour.

3. Process according to claim 1 wherein the food product is a dairy product.

4. Process according to any one of claims 1 to 3 wherein the pigment is a carotenoid.

5. Process according to claim 4 wherein the enzyme is β-carotene 15,15'-monooxygenase (EC 1.14.99.36), 9-*cis*-epoxycarotenoid dioxygenase or β-carotene 9',10'-dioxygenase.

6. Process according to any one of claims 1 to 5 claims wherein the enzyme is added as an enzyme preparation derived from a microorganism or produced in situ by a microorganism capable of producing said enzyme.

7. Process according to claim 6 wherein the microorganism is a bacterium, a fungus or a yeast.

8. A food product obtainable by the process according to any one of claims 1 to 7.

9. Use of enzymes capable of directly converting pigments into a form which results in an increased whiteness of at least part of a food product.

10. Use of enzymes capable of directly converting pigments for household detergents or in enzymatic stone bleach processes.

--ABSTRACT

The present invention relates to a process for the production of a food product whereby an intermediate form of said food product comprises a pigment, which process comprises adding at least one enzyme that is effective in directly converting said pigment into a form which results in increasing the whiteness of at least part of the food product compared to the food product for which said enzyme is not added during its production. The invention also relates to food products obtained from the process of the invention.

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